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**TECHNICAL MANUSCRIPT 132**

**RIFT VALLEY FEVER VIRUS ASSAY  
IN SERUM-FREE  
HAMSTER KIDNEY MONOLAYERS**

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TECHNICAL MANUSCRIPT 132

RIFT VALLEY FEVER VIRUS ASSAY IN SERUM-FREE HAMSTER KIDNEY MONOLAYERS

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# ABSTRACT

High titers of some arboviruses, including Rift Valley fever (RVF) virus, are obtained in cell cultures without any observable cytopathogenic changes. In the absence of serum, however, a rapid destruction of tissue-culture cells occurred with RVF virus. Preparation of virus dilutions in a serum-free maintenance medium and observation of infected tube monolayers has been found to be an effective method for achieving reproducible titrations of RVF virus. It is possible to calculate a median cytopathogenic effect dose (CPED<sub>50</sub>) comparable to titers determined by a plaque technique but more sensitive than in vivo titration. The tube monolayer titration is more economical, more rapid, and simpler to perform than previously used methods.

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#### TITRATION OF RIFT VALLEY FEVER VIRUS IN HAMSTER KIDNEY CELLS IN THE ABSENCE OF SERUM

Multiplication of certain arboviruses in cell cultures may not be accompanied by cytopathogenic changes (cpe) even though high titers may be obtained in extracellular fluids.<sup>1</sup> High titers of Rift Valley fever (RVF) virus also are obtained in various tissue-culture systems in the absence of any cellular changes. Experiments in this laboratory have shown, however, that a rapid destruction of tissue-culture cells by RVF virus occurred when serum was omitted from the medium. This observation suggested a more economical method for the titration of RVF virus than the plaque technique or the mouse titrations presently employed.

The data reported here were obtained with a third tissue-culture passage of a seed of infected-lamb serum of the van Wyk strain of RVF virus. The hamster kidney (HK) cells used in these studies were propagated in a growth medium consisting of medium 199<sup>3</sup> plus 10% calf serum. Culture tubes (16 by 150 mm) were inoculated with one milliliter of cell suspension (500,000 cells per milliliter) and after a 24-hour incubation at 37°C, a confluent sheet of cells was obtained and the growth medium was removed. The cells were inoculated with virus suspended in serum-free maintenance medium and absorption was allowed to proceed for one hour. After the absorption period, fresh maintenance medium (serum-free) was added and the cultures were incubated at 37°C during the observation period.

The maintenance medium was prepared from a concentrated (10X) stock medium 199 diluted to 1X with distilled, demineralized water. The 1X solution was further diluted with one volume of balanced salts solution (BSS) to make a final dilution of 1:2. Virus dilutions were prepared in this serum-free maintenance medium and 0.1 ml of each dilution was inoculated into each of four tube cultures. Although the dilutions of virus for the tube titrations were made in the maintenance medium, the growth medium containing calf serum had to be employed for the corresponding plaque titrations, because comparable titers of virus were not obtained when calf serum was deleted from the agar overlay. Furthermore, the use of serum-free maintenance medium in the plaque technique resulted in a decrease in the number and size of the plaques.

The endpoints of the titrations, based on the cpe, were calculated by the method of Reed and Muench<sup>4</sup> and expressed as a median cytopathogenic effect dose (CPED<sub>50</sub>). Observations for cpe were made daily for ten days; however, final endpoints were usually achieved by the fourth or fifth days. For routine titrations, daily examination of tubes would not be necessary.

CPED<sub>50</sub> titers of RVF virus were directly compared with titers obtained by three other methods of titration: a plaque technique\* and mouse inoculation by either intracerebral or intraperitoneal routes (Table I). The CPED<sub>50</sub> endpoints of the serum-free tube tests compare favorably with results obtained with the plaque technique. This method of titration appears to be as reproducible as the latter but more sensitive than the in vivo titrations. Examinations of the extracellular fluids were negative for virus even in cultures showing extensive cell damage. This may be partially due to the thermal lability of RVF virus in the absence of serum. Although further experiments are required to determine the underlying cause of cell destruction in the absence of serum, it is presently known that the effect is not seen with UV- or heat-inactivated virus, and the cpe can be prevented by specific immune serum.

This method of titration has many advantages over the plaque and in vivo techniques. It is considerably more economical of materials, simpler to perform, and more rapid. These results warrant additional investigations on tube titration in a serum-free medium with other arboviruses that can multiply in cell cultures without producing obvious cell changes.

TABLE I. TITERS OF RIFT VALLEY FEVER VIRUS  
DETERMINED BY VARIOUS TITRATION PROCEDURES

Virus Sample No.	Median Cytopathogenic Effect Dose, CPED <sub>50</sub>	Plaque- Forming Units, PFU	Intra- cerebral	Intra- peritoneal
1	8.3 <sup>a</sup> /	8.5	7.1	6.9
2	7.7	7.8	6.5	6.3
3	8.3	8.0	<sup>b</sup> /	-
4	8.0	8.6	-	-
5	8.0	8.0	7.0	6.0
6	8.5	8.2	7.5	7.0

a. Log<sub>10</sub> per milliliter.

b. Not titrated.

\* J. L. Runnels, unpublished data.

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